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(21) *Patentansökningsnummer*   *0203137-5*
     Patent application number

(86) *Ingivningsdatum*   *2002-10-24*
     Date of filing

Stockholm,   2003-11-13

För Patent- och registreringsverket
For the Patent- and Registration Office

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DRUG TARGET IN CANCER THERAPY

Background of the invention

The present invention relates to a novel drug target in cancer therapy. More closely it relates to use of the cytochrome P450 enzyme CYP2W1 as a drug target for cancer therapy.

Background of the invention

The cytochromes P450 are responsible for the metabolism of endogenous as well as exogenous compounds. In human at present about 80 different P450 genes have been identified of which 55 are functional whereas 25 are pseudogenes. The human P450 forms can be divided into three major groups: i) those in CYP families 5-51 of endogenous importance having usually high affinity for the substrates and being relatively well conserved during evolution, ii) those in CYP families 1-3, with usually less affinity for their substrates, being less conserved evolutionary and which exhibit important genetic polymorphisms and iii) those in family 4 with properties in between the two other groups (see Ingelman-Sundberg (2002) for a review). CYP enzymes in families 1-3 are active in metabolism of exogenous compounds and currently 19 genes encoding active enzymes have been described. The cytochromes P450 in these families are responsible for 70-80 % of all phase I dependent metabolism of clinically used drugs and participate in the metabolism of a huge number of other xenobiotic chemicals. This can lead to the metabolic activation of precarcinogens and drugs, which might exert toxic or carcinogenic effects. This capacity to activate prodrugs to cytotoxic products are seen e.g. in the metabolic activation of acetaminophen which is metabolised by CYP2E1 and CYP3A4 to a reactive imine intermediate that can cause liver hepatotoxicity . Other examples of cytochrome P450 dependent metabolic activation come from prodrugs in cancer therapy where P450 enzymes like CYP2B6 inserted by viral transfection into tumours facilitate tumour suppression upon treatment with cancer drugs agents like e.g cyclophosphamide or ifosfamide (Waxman et al., 1999, Jounaidi and Waxman, 2001).

Summary of the invention

Another approach for drug targeting in cancer therapy would be to utilize a specific form of cytochrome P450 only present in the transformed tissue being able to activate prodrugs converted to cytotoxic products. The present inventors have identified a novel human cytochrome P450 form, CYP2W1, mainly expressed in tumour cells. This finding represents a major step of importance for the development of new potential agents effective in cancer therapy.

Thus, in a first aspect the present invention relates to the use of the cytochrome P450 enzyme CYP2W1 and genetic variants thereof as a drug target in cancer therapy. CYP2W1 was found by the inventors to be selectively expressed in tumor tissues such as lung carcinoma, colon carcinoma and ovarian carcinoma. A common problem in cancer therapy is the unselective toxicity exerted by cytotoxic drugs against cancer cells and their healthy counterparts. The present invention shows the potential to improve drug targeting to tumor cells solely, by administering substances that can be metabolically activated by CYP2W1 to cytotoxic agents. Accordingly, the invention provides a method for screening for such substances, substrates for CYP2W1, which can be modified by the enzyme to obtain a cytotoxic metabolite. A second aspect of the invention relates to the combinatorial use of a substance-substrate-to-be-metabolised, and a substance that can induce the expression of CYP2W1 in the tumor cells, thus resulting in higher concentrations of the cytotoxic metabolite at the site of the tumor.

The invention can for example be used for, but not restricted to, the treatment of lung tumours, colon tumours or ovarian tumours, or all or any tumour expressing CYP2W1 constitutively or after administration of an inducing agent.

In a second aspect, the invention relates to a method of providing therapeutic agents for cancer therapy, comprising screening for such agents by using CYP2W1 as a drug target. The therapeutic agents shall modulate the activity of CYP2W1, such as induce or increase the activity thereof..

In a third aspect, the invention relates to a method of treating cancer, comprising giving a patient in need thereof a therapeutically effective amount of a substance activated by the enzyme CYP2W1 and/or inducing the enzyme CYP2W1.

For example, the substance may be activated to an anticancer agent or a cytotoxic substance.

In order to screen for cytotoxic agents, anticancer agents, or other substrates, activated by CYP2W1, the enzyme is expressed using for example yeast as expression system. Other expression systems like bacterial and mammalian expression system could also be used as enzyme source. The obtained microsomes are incubated with a fluorescent substrate that is metabolised by CYP2W1. Inhibition of that reaction is then used for drug screening with different substrates. Candidate substrates to be tested, but not restricted to, could be drugs with moieties that are known to be metabolised by other members of the CYP2 family. The substrate that shows the best inhibition can be modified in order to obtain a cytotoxic metabolite. Thus the invention relates to a screening assay using CYP2W1 as a drug target.

In order to screen for agents inducing the expression of CYP2W1 different cell lines can be used. One example of possible cell line is the human hepatoma cell line HepG2, which is constitutively expressing CYP2W1. The agent to be tested will be administered to the supplement medium and the induction of the CYP2W1 expression can be tested on the RNA, protein and/or enzymatic activity levels. Candidate agents to be tested, but not restricted to, could be known inducers of cytochrome P450s belonging to the CYP2 and CYP3 family, including those being efficient substrates for the nuclear receptors PXR, LXR, FXR and CAR.

Detailed description of the invention

The present invention will be described in more detail below in an experimental section.

Materials and Methods

Bioinformatics

The BLASTN search algorithm was used to search the Celera sequence databases (http://www.celera.com) for sequences related to the previously reported CYP2W1 partial cDNA sequence (GenBank Accession No. AK000366). The amino acid sequence of CYP2W1 was compared with the amino acid sequences of other human P450s using multiple sequence alignment. An unrooted phylogenetic tree was calculated using ClustalW 1.8 (Thompson et al., 1997) and visualized using the program unrooted (http://pbil.univ-lyon1.fr/software/unrooted.html).

RNA Isolation and 1ˢᵗ strand cDNA Synthesis

Total RNA was isolated from cultured HepG2 and B16A2 cells using the TRIZOLE Reagent (Invitrogen, Rockville, MD) based on the acid guanidine thiocyanate phenol chloroform extraction method, whereas total RNA from HEK293 and HeLa cells was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) Reverse transcriptase was performed using MMLV Reverse Transcriptase (Invitrogen, Rockville, MD) according to the manufacturer's instructions.

Amplification and Sequencing

cDNA from HepG2 cells was used as a template in PCR reactions with CYP2W1 specific primers. The enzyme used for amplification was Taq DNA Polymerase (ABgene, Epsom, UK). The PCR products generated were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA), according to the manufacturers instructions, and analyzed on an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA).

Multiple Tissue cDNA Panels

cDNA from Human Multiple Tissue cDNA (MTC) Panel I and II, Human Digestive System MTC Panel, Human Fetal MTC Panel and Human Tumor MTC Panel (Clontech, Palo Alto, CA) were used as templates in a PCR-reaction using the CYP2W1 specific primer pair, 5'-GAGGTGGAGGCATCTTCTTCTCATCTG-3'/5'-CTGGATCAGGGCGTCCACATAGCTG-3'. A reaction without cDNA was run as a negative control in each experiment. The PCR amplification was performed in the presence of 1 mM MgCl$_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, 0.625 U Taq DNA Polymerase (ABgene, Epsom, UK), 1x Reaction Buffer IV (75 mM Tris-HCl pH 8.8 at 25°C, 20 mM (NH$_4$)$_2$SO$_4$, 0.01% (v/v) Tween 20) and 0.5 ng cDNA. The PCR reaction was performed by an initial denaturation step at 94°C for 3 min, after which the amplification was carried out for the indicated number of cycles, with denaturation at 94°C for 45s, annealing at 60°C for 30s, and extension at 72°C for 1 min and 30s, and a final extension step of 72°C for 10 min. HepG2 cDNA was used as a positive control, and the quality of the cDNA in the MTC panels was ensured by using the G3PDH control primers, supplied by the manufacturer. Amplification was performed under the same conditions as described above. The PCR products were separated and detected on a 1.2% ethidium-bromide stained agarose gel. The

PCR product generated was sequenced and analyzed, as described above, in order to ensure specificity of the reaction.

Real-time quantitative PCR detection

CYP2W1 mRNA expression was quantified in Human Tumor MTC Panel using the Smart Cycler® System (Cepheid, Sunnyvale, CA). The quantitative real-time PCR assay was carried out using the CYP2W1 gene specific primer pair, 5'-AGCTATGTGGACGCCCTGATCCA-3'/5'-ACGCGGTCTAGCTCCTCCTGCAC-3', and SYBR®Green PCR Master Mix (Applied Biosystems, Foster City, CA). The quantification was performed using 0.5 ng of each cDNA sample. To avoid detection of any possible contaminating genomic DNA the primer pair was designed so the resulting product spans exon junctions. Standard curves were constructed with the use of serial 10-fold dilutions ranging from 10^{-2} fmol/μl to 10^{-9} fmol/μl of an accurately determined concentration of a CYP2W1 full-length cDNA fragment. The data was analyzed using the Smart Cycler® Software (Cepheid, Sunnyvale, CA).

Analysis of mRNA by Northern and Dot Blot

A Human Multiple Tissue Expression (MTE) Array (Clontech, Palo Alto, CA) and a Multiple Tissue Northern (MTN) Blot (Clontech, Palo Alto, CA) were hybridized with a ^{32}P-labeled CYP2W1 probe, corresponding to nucleotides 329-986, which was generated by PCR and labeled using the Radprime DNA labelling system (Invitrogen, Rockville, MD). Hybridization was performed according to the manufacturer's instructions.

The ^{32}P-labeled CYP2W1 probe was also hybridized to total RNA from HepG2, B16A2, HEK293 and HeLa cells and total RNA from human liver (provided by Anna Westlind). In particular, 20 μg RNA per lane was loaded on a 1.2% agarose/formaldehyde denaturing gel and subjected to electrophoresis using standard procedures (Struhl, 1993). The RNA was transferred to a Hybond-N+ filter (Amersham Pharmacia Biotech, Uppsala, Sweden) and hybridized using ExpressHyb Hybridization Solution (Clontech, Palo Alto, CA). Prehybridization was carried out at 65°C for 30 minutes, and hybridisation was carried out at the same temperature over night. After hybridisation all three blots were exposed to an imaging plate and analysed by a Bas-1800II phosphoimager using the Image Gauge software (Fujifilm, Samford, CT).

Cell Culture Conditions

HepG2 and HEK293 cells were cultured in Minimum Essential Medium supplemented with 10% fetal bovine serum, 1mM sodium pyruvate, 1% non-essential amino acids, 100 IU/ml penicillin and 100 µg/ml streptomycin. HeLa cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 100 IU/ml penicillin and 100 µg/ml streptomycin. B16A2 cells were cultured in William's E Medium supplemented with 5% fetal bovine serum, 0.6 µM hydrocortisone, 0.25 µg/ml insulin, 100 IU/ml penicillin and 100 µg/ml streptomycin. All cells were cultured in humidified 5% CO_2 at 37°C. All cell culture media and supplements were obtained from Invitrogen, Rockville, MD.

CYP2W1 Antiserum

An 15 amino acid long peptide, TMRPRAQALCAVPRP, corresponding to the C-terminus of CYP2W1 was synthesized and coupled to keyhold limpet hemocyanin (Neosystems, Strasbourg, France). Immunization of two rabbits was carried out with injections at day 0, 14, 27 and 56. Bleedings were performed before the first injection to obtain pre-immune serum, and after 39, 67 and 96 days to obtain the CYP2W1-specific antiserum.

Preparation of Total Cell Extracts and Western Blot

The human cell lines HepG2, HEK293, HeLa and B16A2 were analysed for CYP2W1 expression using western blot. Cells were washed twice, scraped into ice-cold PBS and pelleted by centrifugation at 17,320x g. The pelleted cells were resuspended in a phosphate buffer, containing 50 mM $NaPO_4$ pH 7.4, 0.1mM EDTA and 10% glycerol, and lysed by sonication. After centrifugation at 17,320x g, supernatants were collected and protein concentration was determined according to Lowry. 40 µg total cell extract as well as human liver microsomes and cytosol , were subjected to SDS-PAGE using a 10% gel. The proteins were subsequently transferred to a Hybond-C extra membrane (Amersham Pharmacia Biotech, Uppsala, Sweden). After transfer, the membrane was blocked in TBS containing 0.05% (v/v) Tween 20 and 5% fat-free milk, and incubated with a 1:5000 dilution of the CYP2W1 antiserum. This was followed by an incubation with 1:2000 diluted horseradish peroxidase-conjugated anti-rabbit immunoglobulins and detection using SuperSignal West Pico Chemiluminiscent Substrate (Pierce, Rockford, IL). Blocking experiments were performed using antiserum pre-incubated with

peptide. The CYP2W1 antiserum was incubated together with the peptide used for immunisation, at a concentration of 3 µg peptide per µl antiserum. Incubation was performed at room temperature for 2 hours under gentle agitation. After incubation, the antiserum was diluted and immunoblotting was carried out, as described above.

Construction of CYP2W1 Expression Plasmids

A full-length cDNA clone of CYP2W1 was amplified from cDNA derived from HepG2 cells using Elongase Enzyme Mix (Invitrogen, Rockville, MD). The primer pair used was 5'-GAC<u>AGATCT</u>ATGGCCCTGCTGCTCTTG-3'/5'-GAC<u>TCTAGA</u>CTAGGGCCTGGGCACCGCA-3'. Restriction sites for *Bgl*II and *Xba*I (underlined) were introduced by the primers. The PCR reaction contained 0.2 mM of each dNTP, 0.4 µM of each primer, 2.5 U Elongase Enzyme Mix (Clontech, Palo Alto, CA), and a Buffer A/Buffer B ratio corresponding to 1.7 mM MgCl$_2$ (5x Buffer A containing 300 mM Tris-SO$_4$ pH 9.1, 90 mM (NH$_4$)$_2$SO$_4$, and 5 mM MgSO$_4$; 5x Buffer B containing 300 mM Tris-SO$_4$ pH 9.1, 90 mM (NH$_4$)$_2$SO$_4$, and 10 mM MgSO$_4$). The conditions for the reaction were as follows, a denaturation step at 94°C for 1 min followed by 70°C for 1 min when Buffer A/Buffer B and Elongase Enzyme Mix were added as a hot start. Before amplification an additional denaturation step at 94°C for 1 min was performed. The amplification was carried out with denaturation at 94°C for 30s, annealing at 52°C for 30s, and extension at 68°C for 2 min for 35 cycles, and a final extension step of 68°C for 7 min. The obtained PCR product was digested with *Bgl*II and *Xba*I (Invitrogen, Rockville, MD) and ligated into the expression vector pCMV4, cut with the same restriction enzymes as the PCR product. The sequence inserted was verified by DNA sequencing.

Heterologous Expression in Mammalian Cells

The HEK293 cell line was used for expression of the CYP2W1 protein. The cells were transfected with 30 µg pCMV4-2W1plasmid per 15 cm cell culture dish using Lipofectamine 2000 (Invitrogen, Rockville, MD) according to the manufacturers instructions. Cells transfected with only pCMV4 was used as a negative control. The cells were harvested 66 h after transfection and homogenized in 10 mM Tris/HCl pH 7.4 containing 1 mM EDTA, 0.5 M sucrose and Complete protease inhibitor (Roche, Mannheim, Germany). The homogenate was fractionated by

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centrifugation, and the microsomal fraction was used for estimation of the total P450 content. The microsomal, mitochondrial and cytosolic fractions were subjected to western blot for detection of the CYP2W1 protein, as described above.

Results

The results of the invention will be described below in association with the accompanying drawings.

Figure 1

Gene structure (A), nucleotide and predicted amino acid sequence (B) of CYP2W1. (A) Exons are shown as boxes with size in base pairs. The size of the introns is shown in kilo base pairs. (B) The stop codon is indicated by an asterisk and the proline-rich region, PPGP, and the conserved cysteine are shown in italic bold. The 3'-flanking region was obtained from the Celera Transcript hCT1786066.

Figure 2

Unrooted phylogenetic tree of CYP2W1 and other human P450s belonging to family 1, 2 and 3. The scale bar for distance measurements represents 0.1 amino acid substitutions per site.

Figure 3

Real-time PCR based quantification of CYP2W1 mRNA expression in Human Tumor Multiple Tissue cDNA (MTC) Panel using gene specific primers.

Figure 4

Distribution of CYP2W1 mRNA in different human tissues and cell lines. (A) Multiple Tissue Expression (MTE) Array containing 42-500 ng of normalized human poly(A)$^+$ RNA per dot. (B) Multiple Tissue Northern (MTN) blot containing approximately 1 µg poly(A)$^+$ RNA per lane and Northern blot containing 20 µg total RNA from human liver, HepG2, HeLa, B16A2 or HEK293 cells per lane. All blots were hybridized with a ^{32}P-labeled probe corresponding to nucleotides 329-986 and exposed for 48 hours. The size markers used for the northern blot were the rRNA 28S (4.6-5.3 kb) and 18S (1.8-2.0 kb) band.

Figure 5

Western blot containing four lanes with HepG2 total cell extract corresponding to 40 μg protein per lane. The first two lanes were incubated with CYP2W1 antiserum, while the last two were incubated with antiserum blocked with peptide. The blot was exposed for 3 minutes.

Figure 6

CO-spectral analysis of the microsomal fraction of the pCMV4-2W1 II6 construct.

Figure 7

Western blot analyses of microsomal, mitochondrial and cytosolic fractions obtained from transfected HEK293 cells. Fractions corresponding to 40 μg protein were loaded per lane. The last two lanes contain human liver microsomes and cytosol corresponding to 40 μg protein. The membrane was incubated with CYP2W1 antiserum and exposed for two minutes.

A number of genomic clones related to CYP2W1 were identified and aligned together in order to obtain the *CYP2W1* gene sequence. A Celera transcript, hCT1786066, was also identified which contained the 3' untranslated region of the cDNA. The *CYP2W1* gene was more than 5.5 kb long and showed the typical family 2 gene structure with nine exons (Fig. 1A). The obtained gene sequence was used when designing primers for the amplification and sequencing of the entire HepG2 CYP2W1 cDNA. Sequencing resulted in an open reading frame of 1473 nucleotides, which encodes a 490 amino acid long polypeptide. The nucleotide and predicted amino acid sequence of CYP2W1 are shown in figure 1B. CYP2W1 was found to contain some typical structural features associated with P450s, including a hydrophobic NH_2-terminal, the proline-rich region and the conserved cysteine, which is the fifth heme iron ligand . The CYP2W1 cDNA sequence described here differs extensively from the previously reported partial sequence (GenBank Accession No. AK000366) with respect to the N- and C-terminal exons.

An unrooteed phylogenetic tree of CYP2W1 and other human P450s is shown in figure 2. It shows that CYP2W1 align together with the other family 2 members, but it does not seem to be

closely related to any of them. The enzymes within family 2 with highest identity to CYP2W1 are CYP2D6 (42%) and CYP2S1 (40%).

The tissue-specific CYP2W1 mRNA distribution was analysed by using MTC panels by PCR. The cDNA samples in the MTC panels were pooled from several individuals and normalized with at least four different housekeeping genes. As shown in Fig 3 and Table 1 the tissues that showed the highest expression of CYP2W1 mRNA were the tumor tissues lung carcinoma and colon adenocarcinoma. Moderate expression was seen in for example fetal lung, but the expression levels seen in the majority of normal adult and fetal tissues were low or absent. A rough calculation about the difference in expression between the tumour cells having highest CYP2W1 mRNA and normal tissue reveals a 10,000-100,000 fold difference.

Table 1

Tissue specific expression of CYP2W1 revealed by PCR analyses using gene specific primers and different Human Multiple Tissue cDNA (MTC) Panels. The number of cycles required to obtain a visible product band on an ethidium-bromide stained gel is given in the column. The abbreviation nd stands for not detected, which means that no product band could be seen after 40 cycles of amplification.

Panel	Tissue	Cycles for Detection
Human MTC Panel I	Brain	nd
	Heart	nd
	Kidney	36-40
	Liver	36-40
	Lung	nd
	Pancreas	31-35
	Placenta	36-40
	Skeletal Muscle	36-40
Human MTC Panel II	Colon	31-35
	Ovary	36-40
	Peripheral Blood Leukocytes	nd
	Prostate	31-35
	Small Intestine	nd
	Spleen	nd
	Testis	nd
	Thymus	nd
Human Digestive System MTC Panel	Cecum	36-40
	Colon, Ascending	36-40
	Colon, Descending	36-40
	Colon, Transverse	36-40
	Duodenum	31-35
	Esophagus	36-40
	Ileocecum	36-40
	Ileum	31-35
	Jejunum	26-30
	Liver	31-35
	Rectum	26-30
	Stomach	31-35
Human Fetal MTC Panel	Brain	nd
	Heart	nd
	Kidney	31-35
	Liver	31-35
	Lung	26-30
	Skeletal Muscle	nd
	Spleen	nd
	Thymus	nd
Human Tumor MTC Panel	Breast Carcinoma (GI-101)	nd
	Lung Carcinoma (LX-1)	21-25
	Colon Adenocarcinoma (CX-1)	21-25
	Lung (GI-117)	36-40
	Prostatic Adenocarcinoma (PC3)	31-35
	Colon Adenocarcinoma (GI-112)	36-40
	Ovarian Carcinoma (GI-102)	26-30
	Pancreatic Adenocarcinoma (GI-103)	nd

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The mRNA distribution was also examined using different mRNA blots, like MTE Array, MTN blot and Northern blotting. The mRNA amounts on the MTE Array have been normalized using eight different housekeeping genes, and the MTN blot has been normalized with respect to β-actin. Using these two methods for mRNA analysis no signals, and hence no expression, could be observed in any of the tissues (Fig. 4A and B). A Northern blot analysis was also performed using RNA from HepG2, B16A2, HEK293 and HeLa cells and human liver. In HepG2 cells a strong signal corresponding to a transcript of approximately 2.3 kb was observed (Fig.4B), which corresponds well to the sequence shown in Figure 1B. No signals could be obtained in the other cell lines or in human liver, which is consistent with the low CYP2W1 mRNA expression seen in liver using the MTC panels.

A CYP2W1 antiserum was produced by immunization of rabbits, using the C-terminal sequence of CYP2W1. The cell lines examined for the presence of CYP2W1 using western blotting were HepG2, HEK293, HeLa and B16A2, and in addition, we also determined the content in human liver microsomes and cytosol. The membranes containing HepG2 total cell extract and incubated with CYP2W1 antiserum recognized two bands of approximately 54 and 52 kDa as seen in Figure 5. The upper band is the result of unspecific binding to a cytosolic protein, whereas the size of the lower band corresponds well to the calculated CYP2W1 protein sequence of 490 amino acids. When immunoblotting was made using pre-immune serum (data not shown) or antiserum blocked with the immunizating peptide the previously seen bands were abolished (Fig. 5). No band corresponding to CYP2W1 was seen in human liver or in the other cell lines examined (data not shown). Cross-reactivity with other P450s could be excluded since no band was observed in human liver microsomes, which normally shows high expression of most P450s in families 1-3.

CYP2W1 was heterologously expressed in HEK293 cells using three different pCMV4-2W1 constructs (II6, III6 and III7) and empty pCMV4 vector as a negative control. The microsomal fraction of the transfected cells was used for an estimation of the P450 content by spectral analysis of the reduced CO-bound form (Fig. 6). A peak was observed at approximately 450 nm indicating presence of active P450 enzyme. The amount of P450 obtained was 5 pmol/mg microsomal protein. The microsomal, mitochondrial and cytosolic fractions were subjected to

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western blotting for detection of the CYP2W1 protein (Fig. 7). A strong band indicating CYP2W1 were seen in the microsomal and mitochondrial fractions transfected with all pCMV4-2W1 constructs whereas no bands could be seen in the negative control.

Discussion

Alignment of genomic clones allowed the construction of the gene structure of CYP2W1. Based upon this it was possible to make a full length cDNA construct using mRNA from HepG2 cells which revealed a P450 enzyme with expected length and sequence motifs. Semiquantitative analysis for the amount of mRNA expressed in human MTC panels revealed the highest expression in a lung, a colon and an ovarian tumour, whereas in general only small mRNA expression was seen in preparations from adult tissues. Based upon the PCR number of cycles necessary to amplify the product a rough calculation reveals 10,000-100,000 fold higher CYP2W1 mRNA expressed in these tumour cells than in non-transformed cells. Further quantitative estimations are now done using Northern blot filters from different human tumours. The inventors detected significant mRNA expression in HepG2 cells whereas, by contrast, they could not see any significant expression as determined by Northern blotting in different tissues from adult human.

The inventors successfully obtained a specific antiserum to the protein. Western blotting experiments revealed that no significant expression was seen in human liver, but that high expression was seen in the transformed cell line HepG2. This is promising in order to obtain specific Western blotting analyses for detection of the enzyme in different tumours. In HEK293 cells heterologously expressed with CYP2W1 the expression of three bands was seen with slightly different molecular weights. The significance of this observation is currently evaluated.

Large cultivation of HEK293 cells transfected with pCMV-CYP2W1 cDNA allowed the preparation of microsomes useful for detection of spectrally identifiable CYP2W1. The results showed a typical P450 spectrum indicating proper folding of the enzyme being catalytically active.

The transformed cells line HepG2 has not been described to express any known form of microsomal cytochrome P450 at significant levels. Unexpectedly, the present inventors found relatively high CYP2W1 expression as revealed by the Western blotting experiments in HepG2 cells. The background for this is unknown but current work aims at identifying the transcription factors and cis acting elements required for this expression. In addition, the semiquantitative determination of CYP2W1 in some different human tumours revealed an exceptionally high expression.

The present inventors propose use of the enzyme CYP2W1 as a drug target for the development of cytotoxic drugs aimed for cancer therapy. Previously the extrahepatic CYP1B1 form has been shown to be more abundantly expressed in tumours than in non transformed tissue whereas CYP2W1 appears to represent a much more important example of tumor specific cytochrome P450 expression.

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CLAIMS

1. Use of the cytochrome P450 enzyme CYP2W1 and genetic variants thereof as a drug target in cancer therapy.

2. Use according to claim 1 in the treatment of lung tumours.

3. Use according to claim 1 in the treatment of colon tumours.

4. Use according to claim 1 in the treatment of ovarian tumours.

5. A method of providing therapeutic agents for cancer therapy, comprising screening for such agents by using CYP2W1 as a drug target.

6. A method according to claim 5, comprising screening for therapeutic agents modulating the activity of CYP2W1.

7. A method according to claim 6, comprising screening for therapeutic agents increasing the activity of CYP2W1.

8. A method of treating cancer, comprising giving a patient in need thereof a therapeutically effective amount of a substance activated by the enzyme CYP2W1 and/or inducing the enzyme CYP2W1.

9. A method according to claim 8, wherein the substance is activated to an anticancer agent.

10. A method according to claim 9, wherein the substance is activated to a cytotoxic substance.

ABSTRACT

The present invention relates to a novel drug target in cancer therapy. More closely it relates to use of the cytochrome P450 enzyme CYP2W1 as a drug target for cancer therapy.

Fig. 1A

Fig. 1B

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   1 atggccctgctgctcttgctgttcctgggcctcctggggctctgggggctgctctgcgcctgcgcccaagacccctccccagctgcccgg    9
   1 M  A  L  L  L  L  L  F  L  G  L  L  G  L  W  G  L  L  C  A  C  A  Q  D  P  S  P  A  A  R   3

  91 tggcccccggggcctcgcccgctgccgctcgtcgggaacctgcacttgctgcgtctgtcgcaacaggaccggtccctgatggagctctca   18
  31 W  P  P  G  P  R  P  L  P  L  V  G  N  L  H  L  L  R  L  S  Q  Q  D  R  S  L  M  E  L  S   6

 181 gaacgctacgggccggtgttcaccgtgcacctggggcgccagaagacggtggtgctgacggggttcgaggcggtcaaagaggcgctggcg   27
  61 E  R  Y  G  P  V  F  T  V  H  L  G  R  Q  K  T  V  V  L  T  G  F  E  A  V  K  E  A  L  A  90

 271 ggccccgggcaggagctggccgaccggcctcccatcgccatcttccagctcatccagcgaggtggaggcatcttcttctcatctggggcg   36
  91 G  P  G  Q  E  L  A  D  R  P  P  I  A  I  F  Q  L  I  Q  R  G  G  G  I  F  F  S  S  G  A  12

 361 cgctggaggggctgcccgccagttcacggtgcgtgccctgcacagcctgggcgtgggccgggagccggtggctgacaagattctgcaggag   45
 121 R  W  R  A  A  R  Q  F  T  V  R  A  L  H  S  L  G  V  G  R  E  P  V  A  D  K  I  L  Q  E  15

 451 ctgaaatgcctctctgggcagctggatggctacagaggccggcccttcccgctggccctactgggctgggctccctccaatatcaccttc   54
 151 L  K  C  L  S  G  Q  L  D  G  Y  R  G  R  P  F  P  L  A  L  L  G  W  A  P  S  N  I  T  F  18

 541 gcgctcctcttcggccgccgatttgactaccgggacccccgtgtttgtgtccctgctgggtctcatcgatgaggtcatggtcctcttgggg   63
 181 A  L  L  F  G  R  R  F  D  Y  R  D  P  V  F  V  S  L  L  G  L  I  D  E  V  M  V  L  L  G  21

 631 tcccctggcctgcagctgttcaacgtctacccatggctcggggccctgctccagctgcaccggcccgtcctgcgcaagatcgaggaggtc   72
 211 S  P  G  L  Q  L  F  N  V  Y  P  W  L  G  A  L  L  Q  L  H  R  P  V  L  R  K  I  E  E  V  24

 721 cgtgccattctgaggacccctcctggaggcgcggaggcccccacgtgtgcccggggggacccccgtgtgcagctatgtggacgccctgatccag   81
 241 R  A  I  L  R  T  L  L  E  A  R  R  P  H  V  C  P  G  D  P  V  C  S  Y  V  D  A  L  I  Q  27

 811 caggggacaggggggatgaccccgagggcctgtttgctgaggccaacgcggtggcctgcaccctggacatggtcatggccgggacggagacg   90
 271 Q  G  Q  G  D  D  P  E  G  L  F  A  E  A  N  A  V  A  C  T  L  D  M  V  M  A  G  T  E  T  30

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 481 A  Q  A  L  C  A  V  P  R  P  *                                                          49

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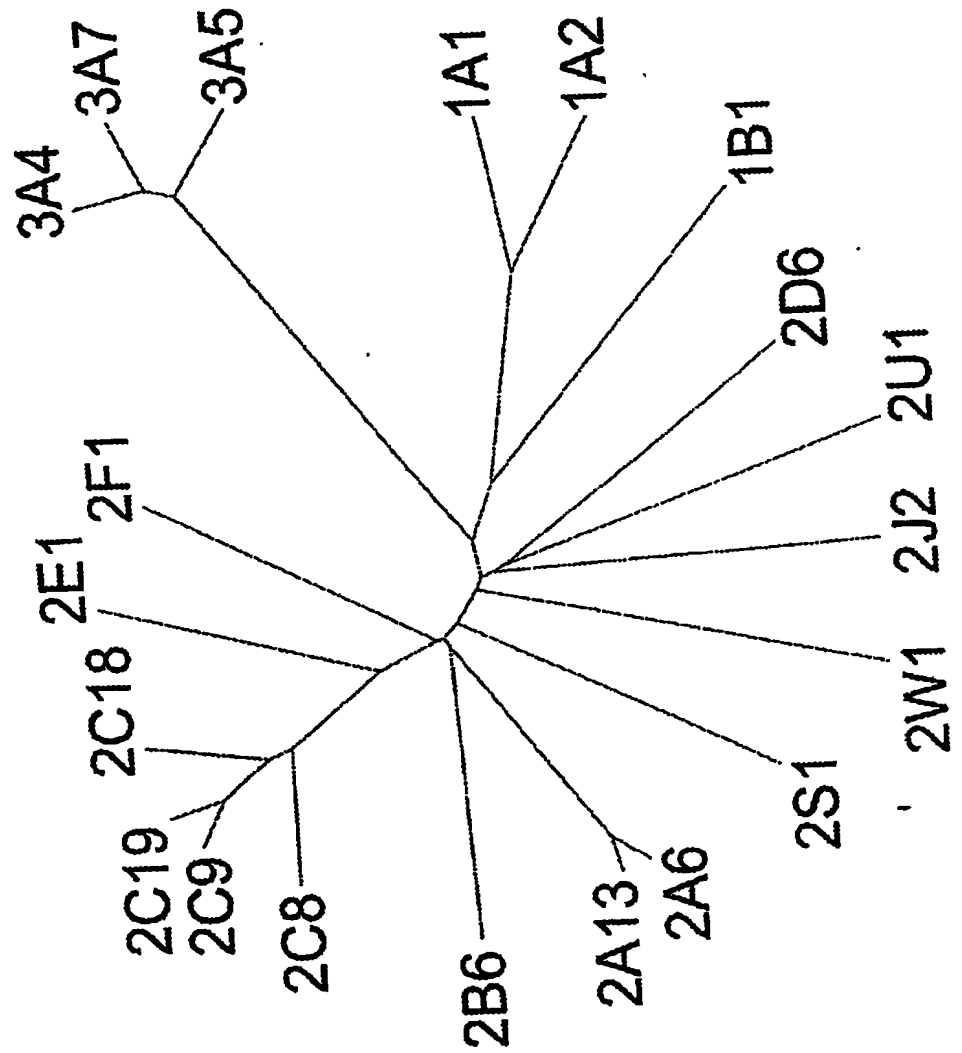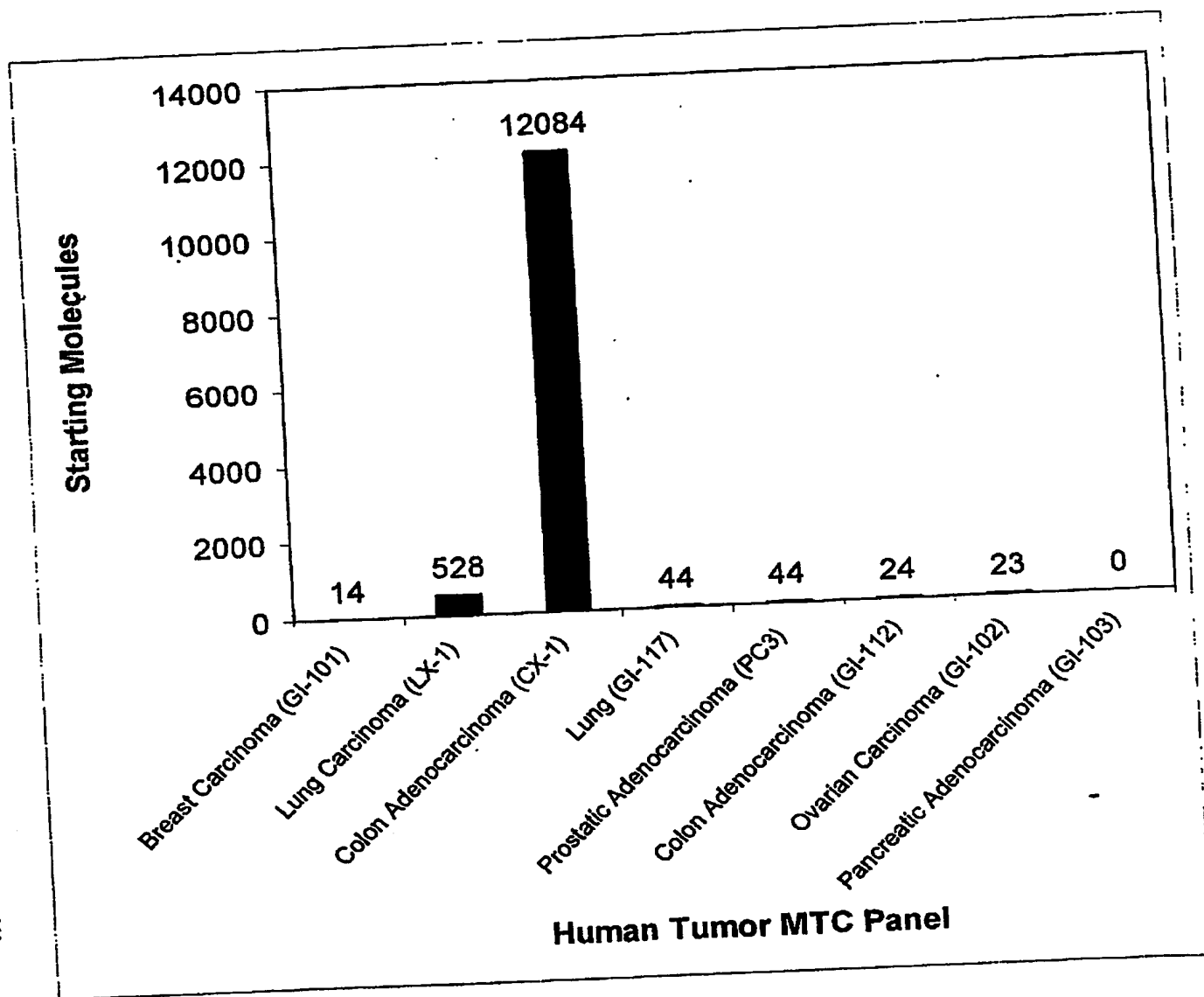
Fig. 2

Fig. 3

Fig. 3 — Bar chart showing Starting Molecules versus Human Tumor MTC Panel. Values: Breast Carcinoma (GI-101) = 14; Lung Carcinoma (LX-1) = 528; Colon Adenocarcinoma (CX-1) = 12084; Lung (GI-117) = 44; Prostatic Adenocarcinoma (PC3) = 44; Colon Adenocarcinoma (GI-112) = 24; Ovarian Carcinoma (GI-102) = 23; Pancreatic Adenocarcinoma (GI-103) = 0.
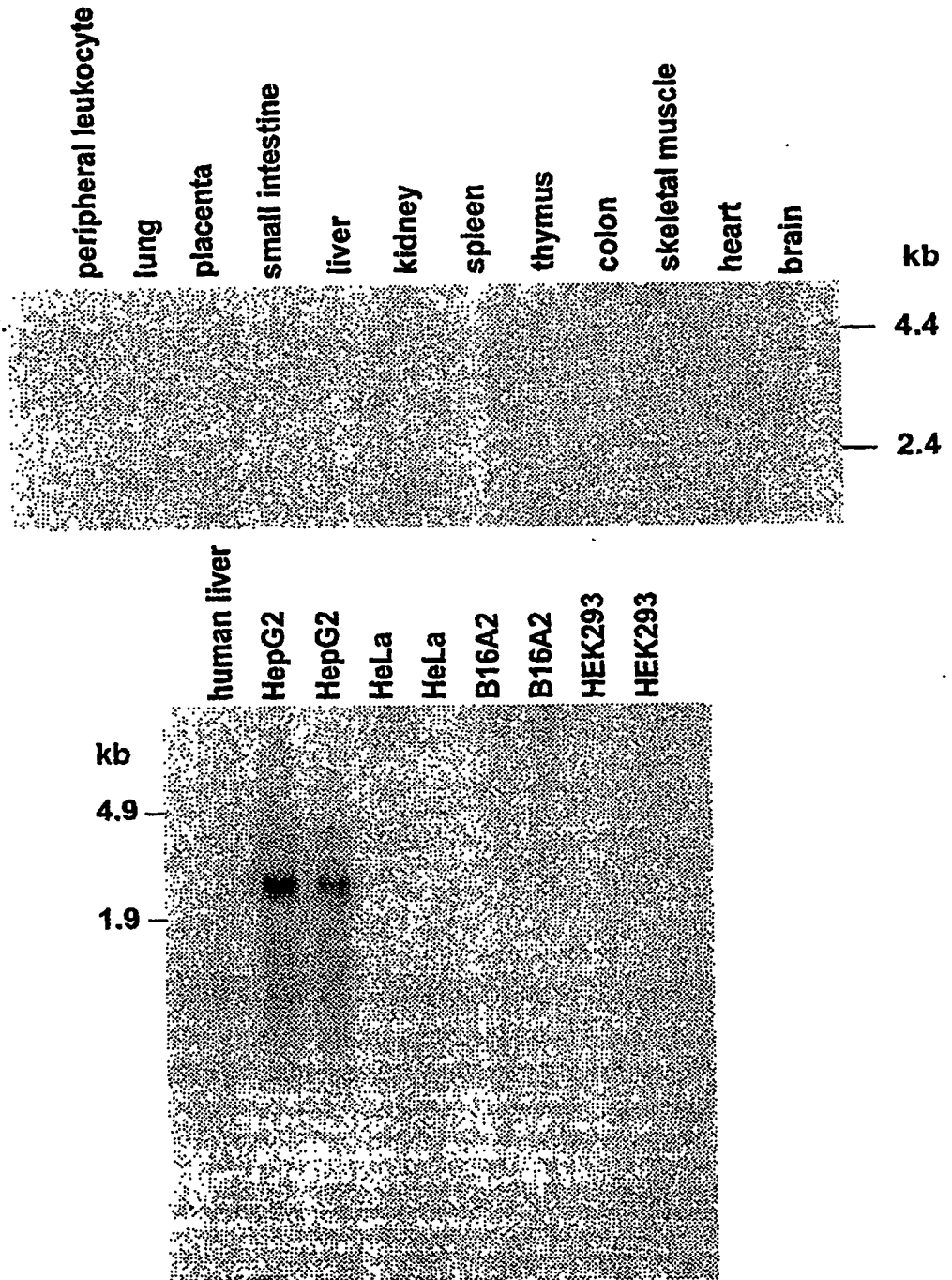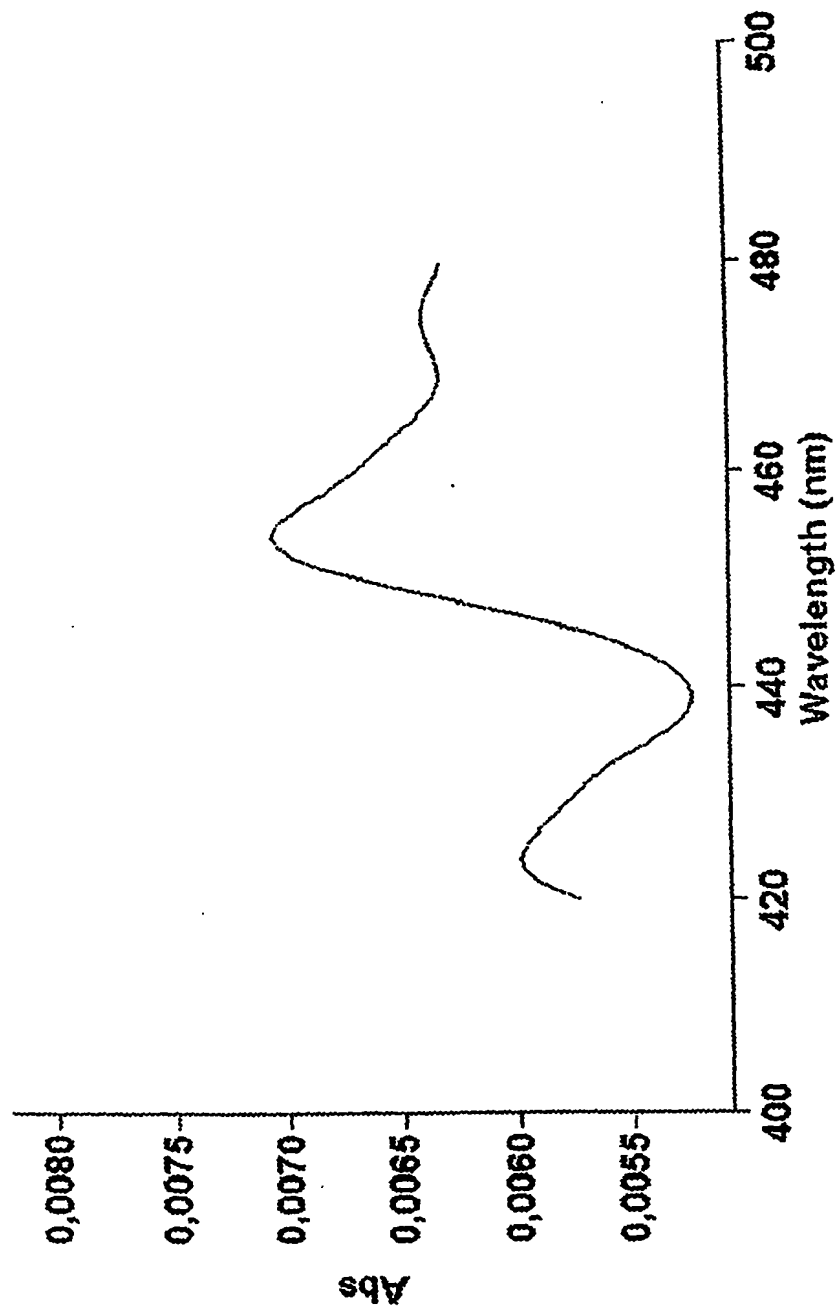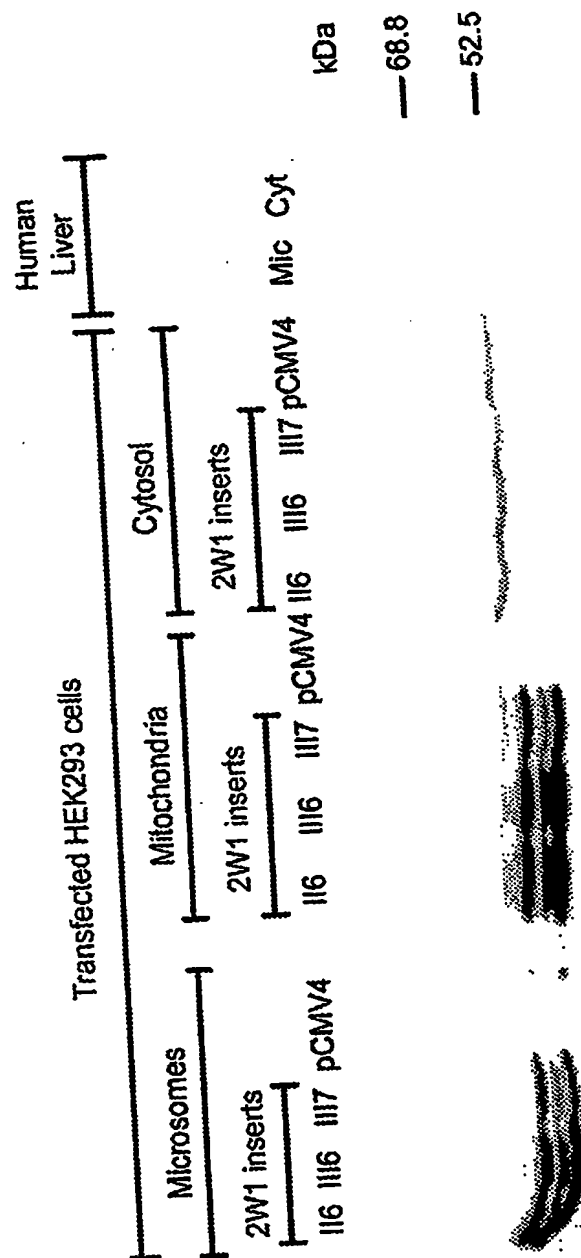
Fig. 4

Fig. 5

Fig. 6

Fig. 7
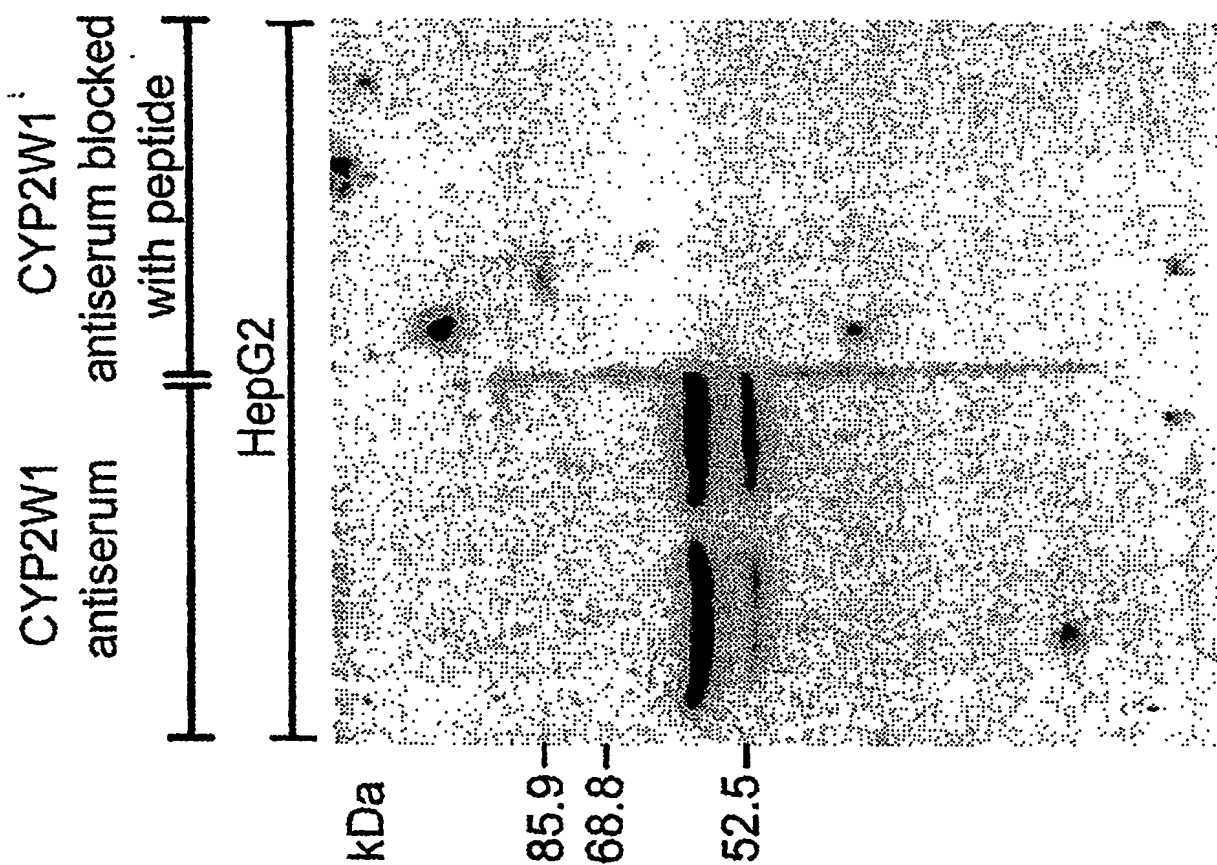
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